ON THE DEVELOPMENT OF *TRYPANOSOMA RHODESIENSE* IN *GLOSSINA MORSITANS*

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In the course of our investigations, we endeavoured to accumulate information regarding the development of the human trypanosome in *Glossina morsitans*. Reference has already been made to this subject in a previous report,* and it is here intended to correlate the facts at our disposal. It may be remarked at once that owing to the comparatively small number of laboratory-bred *Gl. morsitans* available, the information we have collected is by no means so definite as could have been desired.

Up to the present, comparatively little work has been done on this subject, and the records are more or less contradictory. Kleine,† who was the first investigator to write on the development of *T. gambiense* in *Gl. palpalis*, is of the opinion that the complete cycle takes place in, and is limited to, the intestine, whereas the Royal Society Commissioners‡ in Uganda consider that involvement of the salivary glands is essential. They state that without invasion of the salivary glands there is no infectivity of the fly.

* Kinghorn and Yorke. Annals of Tropical Medicine and Parasitology, 1912, Vol. VI.

† Kleine. Trypanosomenstudien. Arb. aus d. Kaiserl. Gesundheitsamte, Bd. XXXI, Heft 2.

‡ Bruce, etc. Reports of the Roy. Soc. Commission in Uganda, 1911.

TECHNIQUE

The method of dissection of the flies used by us was that described by one of us in a previous paper.* Briefly, it consists in splitting the dorsum of the thorax longitudinally, and, after separating the muscles and loosening the tissues with needles, drawing out the salivary glands attached to the pharynx through the waist. This method has obvious advantages over that described by the Royal Society Commission, in which after snipping off the terminal segment of the abdomen, the whole contents were expressed on to a glass slide and the salivary glands subsequently separated from the mass of intestines and other structures. We claim for the technique adopted by us that the process is quicker, more certain, and that the danger of contamination from the intestines is reduced to a minimum. In fact the only lesion in the alimentary canal accompanying the operation occurs in the anterior portion of the oesophagus.

To a certain point the information obtained from our dissections is exceedingly definite. We found that in every fly capable of infecting animals with the human trypanosome (*T. rhodesiense*) the salivary glands were invaded. Of the 160 laboratory-bred *Glossina morsitans* utilised in various experiments to transmit *T. rhodesiense*, 132 were dissected as they died. The remaining 28 were too dry when discovered to allow of dissection. Twenty-seven of those dissected were found to be infected with trypanosomes. The day of the experiment on which the flies died and the results of dissection are given in Table 1.

A glance at the table shows that, of these 132 flies, 5 became capable of infecting animals with the human trypanosome. In each of these there was an enormous invasion of the salivary glands by trypanosomes. In the 127 flies which remained incapable of transmitting the parasite, the salivary glands were not involved, although trypanosomes were found in the intestine of 22.

A precisely comparable state of affairs was observed in dissection of 'wild' *Gl. morsitans* which had become infective after feeding on infected animals. The salivary glands were found to

* Lloyd, Ll. Bull. Entoml. Research, 1912.

TABLE I.—Results of Dissection of Laboratory-bred *Glossina morsitans* which were found to contain Parasites after being fed on Infected Animals.

No.	Date of infecting feed	Day of dissection after infecting feed	Results of Dissection			Remarks
			Proboscis	Intestine	Salivary Gland	
1	9.9.11	4th	o	+++	o	
2	10.8.12	4th	o	+	o	
3	22.1.12	5th	o	+	o	
4	9.8.12	5th	o	++	o	
5	10.8.12	5th	o	+++	o	
6	23.6.12	5th	o	+	o	
7	8.8.12	6th	o	+++	o	
8	9.8.12	6th	o	+++	o	
9	8.8.12	7th	o	+++	o	
10	23.6.12	7th	o	+	o	
11	10.8.12	8th	o	++	o	
12	8.8.12	9th	o	+	o	
13.	10.8.12	10th	o	+++	o	
14	8.8.12	11th	o	+++	o	
15	25.8.11	12th	o	+++	o	
16	31.8.11	12th	o	+++	o	
17	8.8.12	13th	o	+++	o	
18	5.3.12	15th	+	+++	o	
19	11.8.12	19th	++	+++	+++	Infective on 12th day
20	10.8.12	20th	+	+++	o	
21	9.8.12	21st	2 tryps. seen	+++	o	
22	9.8.12	21st	o	+++	o	
23	8.8.12	22nd	1 tryp. seen	+++	o	
24	8.8.12	22nd	+	+++	+++	Infective on 17th to 21st day
25	14.11.11	28th	o	+++	+++	Infective on 15th day
26	22.1.12	29th	o	+++	+++	Infective on 19th day
27	9.9.11	40th	o	+++	+++	Infective on 13th day

N.B. o = Negative, + = Scanty, ++ = Considerable numbers, +++ = Swarming.

TABLE 2.—Results of dissection of Wild *Gl. morsitans* found to be capable of infecting animals with *T. rhodesiense*.

No.	Date of infecting feed	Day of dissection after infecting feed	Result of Dissection			Remarks
			Proboscis	Intestine	Salivary Glands	
1	21.11.11	25th	o	+++	+++	Infective on 11th day
2	21.11.11	25th	o	+++	+++	Infective on 11th day
3	1.7.12	28th	o	+++	+++	Infective on 13th day
4	21.11.11	30th	+	+++	+++	Not proved to be infective, as the monkeys died prematurely
5	21.11.11	30th	+	+++	+++	
6	21.11.11	30th	o	+++	+++	
7	21.11.11	30th	+	+++	+++	
8	14.2.12	39th	o	+++	+++	Infective on 25th day
9	4.10.11	40th	o	+++	+++	Infective in nature
10	30.6.12	42nd	o	+++	+++	Infective on 14th day
11	30.6.12	47th	o	+++	+++	Infective on 14th day
12	13.6.12	58th	o	+++	+++	Infective on 48th day after infecting feed, or 8 days after having been placed in the incubator
13	13.6.12	58th	o	+++	+++	
14	13.6.12	59th	o	+++	+++	
15	14.6.12	71st	o	+++	+++	Not proved to be infective, but inoculation of trypanosomes from gut and salivary glands was followed by positive results

be infected only in those insects which were capable of transmitting the human trypanosome. In all, 906 'wild' *Gl. morsitans* were used in these experiments, and of this number 620 were dissected. The remainder were for various reasons too dry to admit of dissection. Of these 620 flies the salivary glands of 14 were invaded by trypanosomes. All except 4 of these were definitely proved to transmit the human trypanosome. In the case of the other 4, the animals upon which the flies had been allowed to feed died before a diagnosis could be made. None of the 607 flies in which the salivary glands were not involved were able to infect animals with the human trypanosome. Again, the infectivity of

Gl. morsitans in nature was examined, as mentioned in a previous paper, by feeding batches of freshly-caught flies on healthy monkeys. Certain of the groups infected monkeys, and from one of these infective groups the actual infective fly was isolated. This, on dissection, was found to have the salivary glands swarming with trypanosomes. The remaining 242 flies in this group, which had been shown to be non-infective when fed on monkeys, were dissected, and in no instance was an infection of the salivary glands observed.

DISSECTION OF INFECTIVE 'WILD' FLIES

In all, 20 *Gl. morsitans* were found to have invasion of the salivary glands by trypanosomes, and of these 16 were definitely found to be capable of infecting animals with *T. rhodesiense*. Owing to unavoidable circumstances, we were unable to prove the point in the case of the remaining 4, but there is no reason to doubt that, had the animals on which these flies were fed survived beyond the necessary five or six days, they would have proved to be infected.

In order to anticipate the criticism that the trypanosomes were not really inside the salivary glands, but simply lying outside these structures, and due to contamination from the gut, our examinations were conducted with extreme care. In the first place, the glands were removed uninjured and attached to the pharynx, placed on a microscopic slide, and gently covered with a coverslip. By careful focussing it could easily be decided that the parasites were actually in the lumen of the tubes, and not outside. Moreover, they were usually present in such enormous numbers as absolutely to exclude the possibility that they were the result of contamination from the intestine. Again, the glands of other flies were removed with care and immediately fixed, and subsequently imbedded and cut. In the sections the parasites could be seen to be inside the glands. Finally, in order to remove any possibility of doubt, sections of the whole abdomen of these infective flies were made, and the glands found to be loaded with trypanosomes.

It will be seen from Table 1 that it was by no means a rare occurrence for trypanosomes to be present in the intestines in the earlier stages, especially in the case of those flies examined within

a few days of the infected meal. As a general rule, however, most of the insects dissected after the first five or six days were negative. In a certain proportion multiplication of the parasites took place in the intestine.

As to the reason for this multiplication in the gut of occasional flies only, and as to the manner in which it occurs, we have obtained but little information. On one occasion a fly, which died on the twelfth day after having been fed on a guinea-pig infected with *T. rhodesiense*, was found to have an enormous gut infection. Possibly there were also a very few trypanosomes in the salivary glands, but on this point we could not be absolutely certain, as the insect had been dead for some time before the dissection was made. In the mid-gut were found a number of cysts containing swarms of trypanosomes. Some of the cysts had thin walls and were filled with a seething mass of flagellates, while others had thicker walls and the contents were quiescent. The cysts ranged in diameter from 27 to 32μ. Unfortunately, we are unable to state whether the fly was infective at the time of death. It had refused to feed for two or three days previously, and the animal on which it had last fed (ninth day of the experiment) did not become infected. The gut contents were inoculated into a monkey, but the animal died from some unknown cause a couple of days later.

Although multiplication of the parasites occurred in the guts of a proportion of the flies, we met no instance in which a fly was infective and in which inoculation of the gut parasites into experimental animals gave rise to infection unless there was an accompanying invasion of the salivary glands. On the other hand, it appears that on every occasion on which the salivary glands are involved, the trypanosomes, both in these structures and also in the intestines, are virulent, i.e., the fly infects when on a healthy animal, and inoculation of the parasites from either the salivary glands or the intestine gives rise to infection.

The results of inoculation of trypanosomes from laboratory-bred flies in different stages of infection, and also from the wild flies which were proved to be infective, are given in Table 3.

The manner in which the salivary glands become infected is uncertain, but there is a certain amount of evidence which would cause one to believe that it is secondary to the intestinal infection,

Table 3.—Result of inoculation from laboratory-bred *Gl. morsitans* which had been fed on infected animals.

No.	Result of feeding on normal animal the day before the fly was dissected	Day on which dissected after the infecting feed	Result of Dissection			Result of inoculation of trypanosomes from different portions of the fly into clean monkeys or rats
			Proboscis	Intestine	Salivary gland	
1	Negative	4th	o	+++	o	Gut contents; monkey not infected
2	,,	5th	o	+	o	,, ,, ,,
3	,,	8th	o	++	o	,, ,, ,,
4	,,	9th	o	+	o	,, ,, ,,
5	,,	10th	o	+++	o	,, ,, ,,
6	,,	11th	o	+++	o	,, ,, ,,
7	,,	12th	o	+++	o	,, ,, ,,
8	,,	13th	o	+++	o	,, ,, ,,
9	Positive from 12th day onwards	19th	++	+++	+++	Proboscis contents; monkey infected Gut contents; rat infected Salivary glands contents; monkey infected
10	Negative	21st	2 tryps. seen	+++	o	Gut contents; monkey not infected
11	,,	21st	o	+++	o	,, ,, ,,
12	,,	22nd	1 tryp. seen	+++	o	,, ,, ,,
13	Positive from 17th—21st day onwards	22nd	+	+++	+++	Gut contents; monkey infected Salivary glands contents = monkey infected
14	Positive from 15th day onwards	28th	o	+++	+++	Gut contents; monkey infected Salivary glands used for embedding
15	Positive from 19th day onwards	29th	o	+++	+++	Gut contents; monkey infected Salivary gland contents; monkey infected
16	Positive from 13th day onwards	29th	o	+++	+++	Hind gut contents; rat infected Fore gut contents; monkey infected Salivary gland contents; rat infected
17	Positive from 8th day after putting in incubator and 48th day after first infective feed onwards	58th	o	+++	+++	Gut contents; rat infected Salivary gland (right) contents; monkey infected Salivary gland (left) contents; monkey infected
18	(?) *	71st	o	+++	+++	Gut contents; rat infected Salivary gland contents; rat infected

* Owing to the unfortunate death of the monkey on which this fly was fed, we were unable to ascertain whether the insect was infective or not. The fly was one of the series which was kept for 60 days after the infective feed at laboratory temperature, and on the 61st day placed in the incubator. In view of the fact that the parasites both in the salivary glands and in the intestine were infective to sub-inoculated rats, it is highly probable that had the animal, on which the fly was fed, lived long enough, it would have been found to be infected.

and that it only occurs when the trypanosomes in the gut have reached a certain stage of development, and only then when the conditions of temperature are suitable for the further development of the parasites. In the first place, of 752 flies dissected at various intervals after having fed on infected animals, we never found trypanosomes in the salivary glands in the earlier stages before the flies were infective. Again, whenever trypanosomes were found in the salivary glands there were also enormous numbers present in the intestine. Moreover, it is significant that whenever trypanosomes were found in the salivary glands they were always infective, as were also those present in the gut.

Attention has already been drawn in a previous report to experiments which suggest that although the parasites can multiply and develop up to a certain stage in the intestine at comparatively low temperatures (55°—65° F.), yet the flies do not become infective until the temperature to which they are subjected is raised to at least 75°—80° F. In none of our experiments were trypanosomes found in the salivary glands of flies which had not been subjected to the higher temperatures. Probably the salivary glands become invaded by parasites which have reached a certain stage in their developmental cycle in the intestine. The remarkably short period (eight days or less) in which three flies, which had been kept forty days after the infecting feed at laboratory temperature, became infective after being placed in the incubator at 85° F. can be best explained on the assumption that some portion of the cycle must have occurred in the gut during the first forty days at laboratory temperature.

In conclusion, we might remark that invasion of the salivary glands was only observed in the case of flies infected with the human trypanosome (*T. rhodesiense*), and not in the case of any of the other trypanosomes with which we had to deal in either the Luangwa Valley or on the Congo-Zambesi watershed. This was the case both with the strain of *T. rhodesiense* derived from man, and also with that found in 'wild' *Gl. morsitans* which had been infected in nature.

It will be observed that trypanosomes were found occasionally in the proboscis of both infective and non-infective flies. We do not believe, however, that the presence of the parasites in this

structure has any special significance, but rather that it is fortuitous, depending on the passage of the infected salivary secretion, or due to regurgitation from the gut resulting from manipulation during dissection.

It is of interest to note that of 310 'wild' *Gl. morsitans* which were dissected as they were brought into the laboratory, recognisable mammalian red corpuscles were found in the intestine of 70, whilst nucleated red corpuscles were only found on 4 occasions.

MORPHOLOGY OF THE TRYPANOSOME IN *GLOSSINA MORSITANS*

A description of the parasite as it appears in different portions of the tsetse fly must be left for a further communication. It may be stated, however, that the predominant type of the parasite in the salivary glands is quite different from that found in the various portions of the intestine. The form occurring in the salivary glands approximates rather closely to the short variety of the trypanosome in the mammalian blood; nevertheless it is not identical with this. The predominant type in the intestine is a large broad flagellate; the undulating membrane is feebly developed, and there is little, if any, free flagellum. The nucleus is usually fairly central in position, but not infrequently it lies in a more posterior position.

NGOA, NORTH RHODESIA,

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